# A novel BRCA1-associated protein-1 isoform affects response of mesothelioma cells to drugs impairing BRCA1-mediated DNA repair

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DOI: https://doi.org/10.1016/j.jtho.2017.03.023

BRIEF REPORT

# A Novel BRCA1-Associated Protein-1 Isoform Affects Response of Mesothelioma Cells to Drugs Impairing BRCA1-Mediated DNA Repair

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Received 12 September 2016; revised 8 March 2017; accepted 21 March 2017
Available online - 4 April 2017

**ABSTRACT**

**Introduction:** BRCA1 associated protein1 (BAP1) is a tumor suppressor involved in multiple cellular processes such as transcriptional regulation, chromatin modification by deubiquitinating histone 2A, and DNA repair. BAP1 mutations are frequent in malignant pleural mesothelioma (MPM). Our aim was to functionally characterize a newly identified isoform of BAP1 and investigate the effects of its expression on drug sensitivity in MPM.

**Methods:** Expression of BAP1 isoforms was detected by quantitative polymerase chain reaction in MPM and normal mesothelium cell lines and tumor and nontumor samples. Histone H2A ubiquitination levels were analyzed by Western blot after acidic extraction of core histones. Subcellular localization of BAP1 isoforms was examined by immunofluorescence. MPM cell survival in response to poly(adenosine diphosphate–ribose) polymerase (PARP) and dual phosphoinositide 3–kinase (PI3K)–mammalian target of rapamycin (mTOR) inhibitors was analyzed by in vitro assays.

**Results:** We have identified a novel alternative splice isoform of BAP1 (BAP1Δ) that misses part of the catalytic domain. Cells transfected with BAP1Δ showed reduced deubiquitinating activity compared with full-length BAP1. The expression of BAP1Δ transcript is more abundant in nontumor than in tumor samples. MPM cell lines expressing more than 20% of BAP1Δ are more sensitive to olaparib (a PARP1 inhibitor) cytotoxicity, and this sensitivity is enhanced when olaparib treatment is combined with GDC0980 (a dual PI3K-mTOR inhibitor), which induces downregulation of BRCA1.

**Conclusions:** These observations suggest that BAP1Δ does regulate DNA damage response and influences drug sensitivity. It might therefore be relevant to investigate whether patients with high expression of BAP1Δ may be responsive to PARP/PI3K-mTOR inhibitors.



*Keywords:* BRCA1 associated protein 1; Mesothelioma; PARP inhibition; BRCA1

## Introduction

BRCA1-associated protein-1 (BAP1) loss-of-function mutations have been described as one of the main drivers in the complex pathogenesis of malignant pleural mesothelioma (MPM).[1–4] Germline loss of BAP1 leads to a predisposition for different types of cancer, including mesothelioma.[5] BAP1 was initially identified in lung cancer cell lines as a protein that binds to BRCA1, DNA repair associated (BRCA1),[6] which is a well-known

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*Disclosure: The authors declare no conflict of interest.*

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ISSN: 1556-0864
http://dx.doi.org/10.1016/j.jtho.2017.03.023

tumor suppressor that is involved in maintenance of genomic stability and participates in DNA repair of double-strand breaks (DSBs).[7,8] BAP1 is a 90-kDa nuclear-localized deubiquitinating enzyme with ubiquitin carboxyl hydrolase (UCH) activity, and it is the only member of the UCH family with two nuclear localization signal motifs.[9] BAP1-mediated tumor suppression requires both deubiquitinating activity and nuclear localization of BAP1.[9] BAP1 in a complex with the polycomb group protein additional sex combs like 1, transcriptional regulator is essential for histone 2A (H2A) deubiquitination,[10] resulting in chromatin modification. BAP1 loss-of-function mutations have been described as sensitizing cancer cells to ionizing radiation and poly(adenosine diphosphate–ribose) polymerase (PARP) inhibition,[11,12] indicating a role of functional BAP1 in promoting DSB repair.

While investigating BAP1 transcripts in MPM cell lines, we identified by serendipity a novel splice variant of BAP1 (BAP1Δ) missing part of the catalytic domain.

The expression of splice variants is not unusual in cancer because it often offers a survival advantage.[13] We have previously described[14] alternative splicing events occurring in neurofibromin 2 (NF2), another frequently mutated tumor suppressor with a driver role in this disease.[15–19] NF2 transcripts undergo alternative splicing, generating multiple isoforms.[20] Isoform I, which is missing exon 16, and isoform II, which contains all 17 exons, are the two predominant species. However, only isoform I functions as a tumor suppressor and its activity is phosphorylation dependent.[21,22] We have observed that 4% of tumor samples displayed isoform II only. Additionally, the splicing variant delE2/3, which is functionally associated with tumor development in transgenic mice if overexpressed in the absence of wild-type (WT) NF2,[23] was expressed in 35% of the MPM samples and in one sample of the inflammation and normal pleura.

In this study, our aim was to functionally characterize BAP1Δ and investigate whether its expression could be exploited for therapy.

## Material and Methods

### Cell Culture

The mesothelioma cell lines ZL55, ZL34, SPC111, MSTO-211H, ACC-Meso-1, and Mero8 and SDM104 normal mesothelial cells were cultured as previously described.[24] HEK293 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin. Transfected ZL55 cells were selected with puromycin. The breast cancer cell line MCF-7 was kindly provided by Dr. Stucki (Clinic for Gynecology, University Hospital Zürich, Zürich, Switzerland) and maintained in Roswell Park Memorial Institute medium, 10% FCS, and 1% penicillin/streptomycin. All cells were kept at 37°C in a humidified 5% $CO_2$ incubator.

### Tumor Specimens

Tumor specimens were obtained for diagnostic purposes before chemotherapy or at the time of resection.[25] Normal pleural tissue was received from patients undergoing a mesothelioma-unrelated thoracic operation.[25] The study was approved by the University Hospital Zürich ethics committee and written informed consent was obtained from all patients. Twenty RNA samples were selected on the basis of BAP1 determined to be WT by using next-generation sequencing (Oehl and Wild, Institute of Surgical Pathology, Zürich University Hospital, manuscript in preparation) and being representative of the three MPM histologic forms: 16 of 20 epithelial (80%), three of 20 biphasic (15%), an one of 20 sarcomatoid (~1%).

### Reagents

Dulbecco's modified Eagle's medium–F12 medium and penicillin/streptomycin 100x stock solution were purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). Trypsin–ethylenediaminetetraacetic acid 0.25% 1× solution and Opti-MEM medium were purchased from Gibco (Life Technologies Europe, Zug, Switzerland). FCS (CVFSVF00-01) and puromycin were purchased from Eurobio (Les Ulis, France) and from AppliChem (Darmstadt, Germany), respectively. Olaparib (AZD2281, Ku-0059436) was purchased from Selleckchem (Luzern, Switzerland), and GDC-0980 was obtained from Genentech (Roche, Basel, Switzerland). DMRIE-C was purchased from Invitrogen (Carlsbad, CA).

### RNA Extraction, Complementary DNA Synthesis, and Quantitative PCR

Total RNA (0.5 μg) extracted from cells, nontumoral tissue, tumors, or a pool of 12 spheroids with the RNeasy Isolation Kit (Qiagen, Hilden, Germany) was reverse-transcribed with the Quantitect Reverse Transcription Kit (Qiagen). Quantitative polymerase chain reaction (PCR) was performed as previously described.[14] The primers are listed in Supplementary Table 1.

### Protein Extraction and Western Blotting

Total protein extracts were obtained by lysing the cells with assay buffer (Sigma Aldrich) containing 50 mM Tris-HCl, pH 8.0, with 150 mM sodium chloride, 1.0% Igepal CA-630 (NP-40), 0.5% sodium

deoxycholate, and 0.1% sodium dodecyl sulfate (SDS) supplemented with additional SDS to a final concentration of 1% and protease inhibitors, passing it through a syringe and keeping it in ice for 15 minutes. Core histones were obtained by acidic extraction as previously described.[26] Cytosolic and nuclear protein extracts were isolated by using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Pierce Biotechnology, Waltham, MA) according to the manufacturer's instructions and nuclear versus cytosolic purity controls were assessed by probing with anti-PARP (Cell Signaling Technology, Danvers, MA) and anti-vinculin antibodies, respectively. Protein concentration was determined by using the Bradford protein assay and proteins were prepared by adding 6× reducing Lämmli buffer and boiling for 5 minutes. Five or 10 $\mu$g of total proteins or 0.5 $\mu$g of purified histones were separated on denaturing 4% to 20% gradient SDS–polyacrylamide gel electrophoresis gels, 3% to 8% gradient precast acrylamide, or 15% SDS–polyacrylamide gel electrophoresis gels depending on the target and transferred onto 0.45-$\mu$m polyvinylidene fluoride transfer membranes (Perkin Elmer, Schwerzenbach, Switzerland). The membranes were probed with the following primary antibodies: mouse anti-BAP1 (C4), rabbit anti-histone H2A, mouse anti-histone H3, mouse anti-$\gamma$H2AX, mouse anti-$\beta$-actin, rabbit anti-poly(adenosine diphosphate–ribose), rabbit anti-phospho-S6 (Ser235/236), mouse anti-S6, and mouse anti-BRCA1. As a positive control for phospho-S6 (Ser235/236), cell extracts from Mero-83 cells[24] were used. Membranes were then incubated with the secondary antibody goat anti-mouse immunoglobulin G–horseradish peroxidase from Ancell (Bayport, MN) and goat anti-rabbit immunoglobulin G–horseradish peroxidase from Cell Signaling Technology. The signals were detected by enhanced chemiluminescence (ECL Western Blotting Reagents, GE Healthcare, Glattbrugg, Switzerland) and detected on photosensitive film (Super RX Fuji X-Ray Film, Fujifilm, Düsseldorf, Germany). Proteins were quantitated with densitometry by using Image J software, version 1.42q (National Institutes of Health, Bethesda, MD). The percentage of nuclear BAP1 was calculated in single complementary DNA (cDNA)-transfected cells as follows. Nuclear versus cytosolic BAP1, either BAP1Δ or BAP1FL, was normalized to PARP versus vinculin, respectively, and their sum was considered as total BAP1. Nuclear BAP1 was calculated as the fraction of total BAP1. In addition, because in coexpression experiments we observed the accumulation of high-molecular forms of BAP1, we quantified all the bands with a molecular weight higher than that of BAP1Δ and normalized them to PARP versus vinculin, respectively, to estimate their accumulation in the nuclear versus the cytosolic fraction.

### BAP1 Cloning, Sequencing, and Transfection

Human BAP1 and BAP1Δ cDNA amplified from MPM cell lines were subcloned into the Not*I* site of the pCI-puro vector, which contains a puromycin resistance gene,[27] and inserts were validated by sequencing and deposited in Addgene (68365 and 68366, respectively). All primers are indicated in Supplementary Table 1. HEK293 cells were transfected with control vector, pCI-Puro_BAP1FL, or pCI-Puro_BAP1Δ by using DMRIE-C or Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instruction. To test the interaction between the two BAP1 isoforms, ZL55 cells stably expressing control vector or pCI-Puro_BAP1FL were transiently transfected with increasing amounts of pCI-Puro_BAP1Δ.

### Clonogenic Assay

Colony formation assays were performed as follows: 200 cells were plated in six-well plates and subjected to treatment with different concentrations of a specific drug after 1 and 5 days. After an additional 5 days, cells were stained with crystal violet and colonies were counted by eye.

### Immunofluorescence

ZL55 cells transfected with BAP1FL or BAP1Δ were fixed for 10 minutes with 4% paraformaldehyde, permeabilized with 0.1% TX-100 for 10 minutes, and blocked with phosphate-buffered saline containing 1% bovine serum albumin and 1% normal goat serum before incubation overnight at 4°C with Bap1 antibody. Alexa Fluor 488 goat anti-mouse (Life Technologies) antibody was then added for 1 hour at room temperature. Coverslips were mounted using Prolong Gold antifade reagent containing 4,6-diamino-2-phenylindole (Life Technologies). Images were acquired using a Leica DM6000 confocal microscope (Leica Biosystems, Nussloch, Germany).

### Spheroid Formation and Viability Assay

Spheroid formation assays were performed as previously described.[24] At day 4 after seeding, the spheroids were treated with different drugs for 6 days and viability was analyzed using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Dübendorf, Switzerland) to determine the adenosine triphosphate (ATP) content. Luminescence was acquired by using a GloMax 96 Microplate Luminometer (Promega). Each experiment was performed in triplicate.

### Phylogenetic Analysis

Multiple alignments of the protein sequences have been performed through MAFFT FFT-NS-i (v7.215) (http://mafft.

cbrc.jp/alignment/server/). A phylogenetic tree was created with Molecular Evolutionary Genetics Analysis software and the following sequences were obtained from the National Center for Biotechnology Information: *Homo sapiens* (reference sequence [ref seq] XM_017007303.1), *Pongo abelii* (ref seq XM_009238935.1), *Mandrillus leucophaeus* (ref seq XM_011986223.1), *Colobus angolensis palliates* (ref seq XM_011958849.1), *Chlorocebus sabaeus* (ref seq XM_007984488.1), *Rhinopithecus roxellana* (ref seq XM_010365018.1), *Tupaia chinensis* (ref seq XM_006169325.2), *Pteropus vampyrus* (ref seq XM_011372563.1), *Pteropus alecto* (ref seq XM_015593797.1), *Bos taurus* (ref seq NM_001102549.1), *Ovis aries* (ref seq XM_012099955.2), and *Loxodonta africana* (ref seq XM_010589758.1).

### *Statistical Analysis*

Statistical analysis was performed with GraphPad Prism software, version 5.04 (GraphPad Software, La Jolla, CA). Data are presented as the average plus or minus SD. To determine significant additive effects between combination treatments and inhibition of cell death with the inhibitors, an analysis of variance test was performed. Differences with a $p$ value less than 0.05 were considered significant.

## Results

### *Identification and Characterization of a Novel BAP1 Splice Variant*

While investigating BAP1 transcripts in MPM cell lines, we identified a shorter variant (BAP1Δ) missing 54 base pairs within exon 9 compared with the full-length BAP1 (BAP1FL) (Fig. 1*A*). On the basis of data on the crystal structure of the UCH domain (https://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?ascbin=8&maxaln=10&seltype=2&uid=187738&querygi=508123781&aln=4,10,0,32,42,33,63,106,96,51,157,150,69), BAP1Δ is missing 12 amino acids of the catalytic region and part of the BARD1 binding domain and has been already listed as BAP1 transcript in the Ensembl database. It may result from a second acceptor site (http://www.umd.be/HSF3/index.html), which is almost as strong as the one for

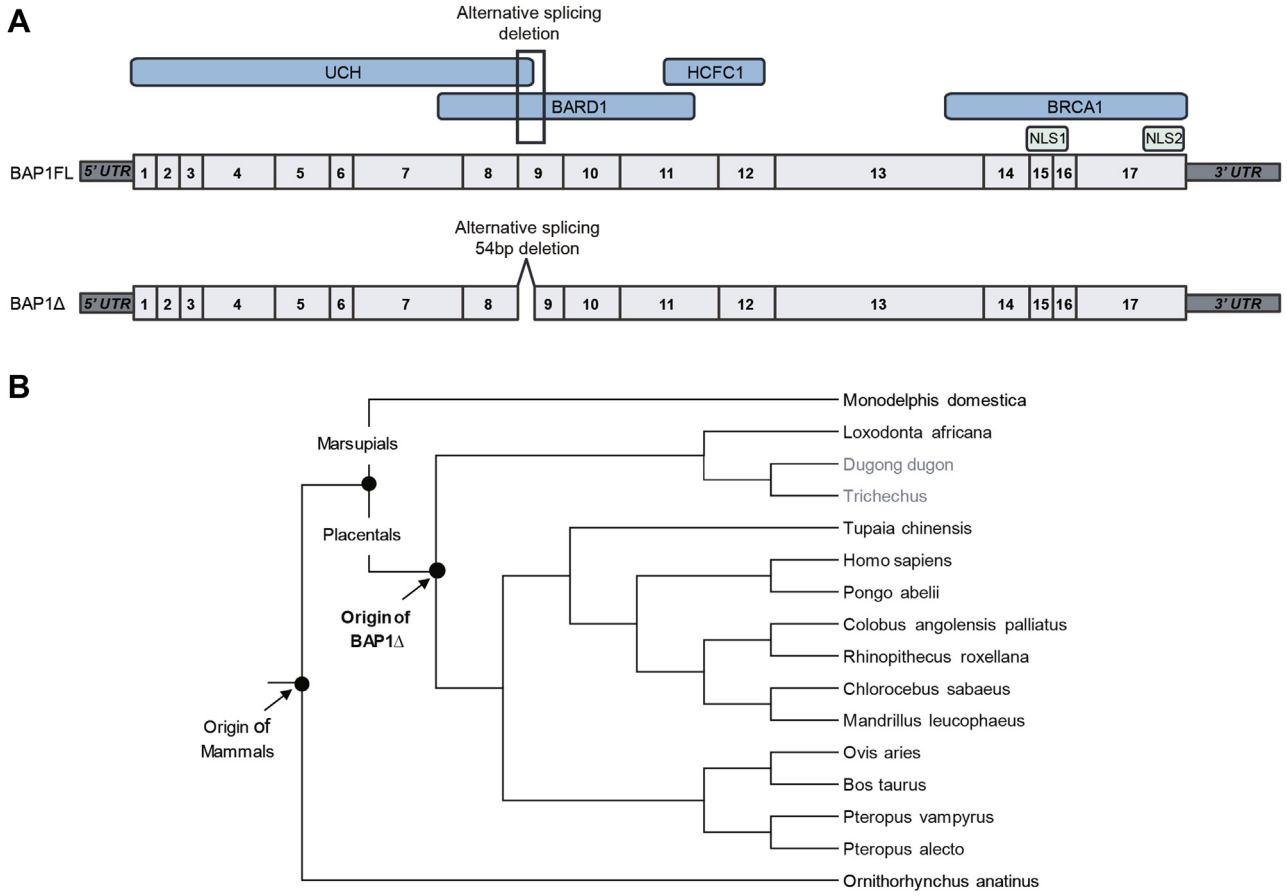


**Figure 1.** Identification of a novel BRCA associated protein1 (BAP1) splice variant (BAP1Δ). (*A*) Schematic representation of a coding sequence and protein domains of full-length BAP1 (BAP1FL) and BAP1Δ. (*B*) Phylogenetic tree of the protein sequences of BAP1Δ. The phylogenetic analysis indicated that the first appearance of BAP1Δ dates back to approximately 150 million of years ago. UCH, ubiquitin carboxyl hydrolase; BARD1, BRCA1 associated RING domain 1; HCFC1, host cell factor C1; BRCA1, BRCA1, DNA repair associated; UTR, untranslated region; NLS, nuclear localization signal; bp, base pair.

BAP1FL. BAP1Δ is present in several species, and multi-species sequence alignment and phylogeny analysis revealed that BAP1Δ appeared first in placentals, dating back to approximately 150 million years ago (Fig. 1*B* and Supplementary Data 1). A fragment covering the region of alternative splicing was amplified by PCR, which revealed the expression of both BAP1FL and BAP1Δ in MPM (Fig. 2*A*). Using a set of primers that allows detection of BAP1Δ specifically, we detected the BAP1Δ isoform in both MPM cell lines and tumors (Fig. 2*B*). Interestingly, BAP1Δ was less abundant in tumors expressing WT BAP1 than in nontumor samples (Fig. 2*C*).

HEK293 cells transfected with BAP1FL (Fig. 3*A*) showed a reduction of monoubiquitinated H2A, whereas no change was observed in the cells expressing BAP1Δ (Fig. 3*B* and Supplementary Fig. 1), confirming that the deubiquitinase activity of this splice variant is impaired, which is consistent with our prediction based on the sequence. We transfected BAP1-deficient ZL55 mesothelioma cells with BAP1FL or BAP1Δ; then, using immunofluorescence analysis, we determined that BAP1Δ localizes to the nucleus, however, not to the same extent as BAP1FL (Fig. 3*C*). This observation was confirmed in experiments in which ZL55 cells stably expressing control vector or pCI-Puro_BAP1FL were transiently transfected with increasing amounts of pCI-Puro_BAP1Δ, after which cytosolic versus nuclear fractions were isolated. Addition of BAP1Δ resulted in increased levels of high-molecular-weight bands with BAP1 immunoreactivity in total lysates (Fig. 3*D*). Quantification of cellular fractionation experiments in single cDNA-expressing cells revealed a 53% plus or minus 4% decrease in nuclear accumulation of BAP1Δ compared with BAP1FL, as estimated by comparing the percentage of normalized BAP1 immunoreactivity present in the nucleus (Fig. 3*E* and Supplementary Fig. 1). In cells coexpressing BAP1FL and BAP1Δ, the quantification of BAP1FL as the sum of molecular forms higher than BAP1Δ (see Fig. 3*E*) indicated a shift in BAP1 distribution from nucleus to cytosol (Fig. 3*F*).

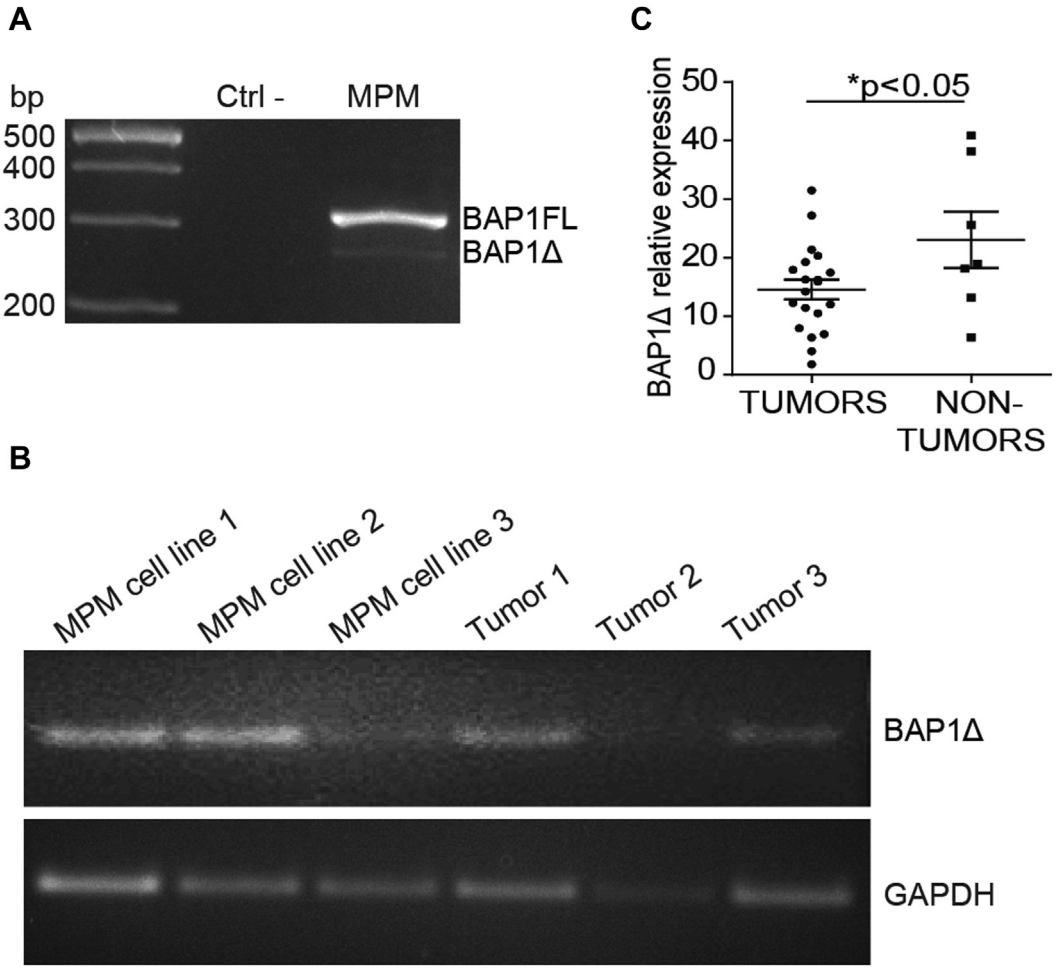


**Figure 2.** Characterization of BRCA associated protein1 (BAP1) splice variant (BAP1Δ). Detection of full-length BAP1 (BAP1FL) and BAP1Δ in a representative malignant pleural mesothelioma (MPM) cell line (*A*) and different level of BAP1Δ expression in MPM cell lines and tumor samples (*B*). (*C*) Quantitative polymerase chain reaction of BAP1Δ in wild-type BAP1-expressing tumors and nontumor samples. Ctrl, control; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

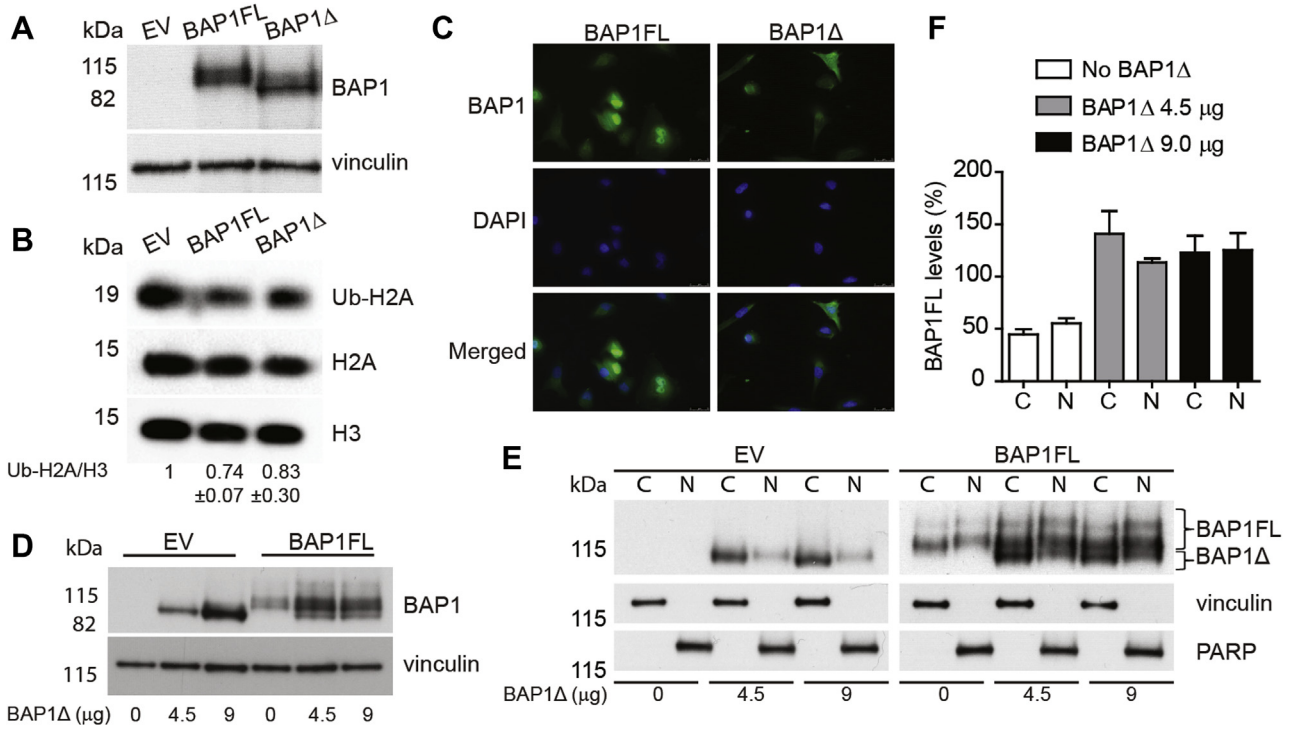


**Figure 3.** Defective deubiquitinating activity and nuclear localization of BRCA associated protein1 (BAP1) splice variant (BAP1Δ). (*A*) HEK293 cells were transiently transfected with empty vector (EV) or with full-length BAP1 (BAP1FL) and BAP1Δ cDNA. (*B*) Core histones were isolated by acidic extraction[26] and Western blot was performed to detect monoubiquitination levels of histone H2A (K119Ub-H2A). Results are representative of three independent experiments. (*C*) ZL55 cells transfected with BAP1FL and BAP1Δ cDNA were analyzed for BAP1 immunoreactivity by immunofluorescence. ZL55 cells expressing EV or BAP1FL were transfected with increasing amounts of BAP1Δ cDNA (*D*) and BAP1 subcellular distribution was analyzed (*E*). Parentheses indicate bands considered as BAP1FL and BAP1Δ in the quantification of nuclear versus cytosolic fraction. (*F*) Quantification of cytosolic and nuclear BAP1FL (including high-molecular-weight forms) in the absence or presence of coexpression of BAP1Δ (n = 3). DAPI, 4,6-diamino-2-phenylindole; C, cytosolic extract; N, nuclear extract; PARP, poly(adenosine diphosphate-ribose) polymerase.

### *Role of BAP1Δ in MPM Sensitization to PARP Inhibitor*

To assess the relevance of BAP1Δ expression in MPM, we used transfected ZL55 cells to assess cell survival after treatment with olaparib, a drug known to be more potent in BAP1-deficient cells.[11,12] Basal levels of protein poly adenosine diphosphate–ribosylation (PARylation), which is indicative of PARP activity were detected, which was consistent with what had been already observed in glioma spheroids,[28] and they decreased after olaparib treatment (Fig. 4*A*). We could observe a twofold to threefold increase in cytotoxicity of the drug in BAP1Δ-transfected cells compared with in BAP1FL-transfected cells in both clonogenic (Fig. 4*B*) and spheroid viability assays (Fig. 4*C*).

### *Both Levels of BAP1Δ and GDC0980-Mediated BRCA1 Depletion Sensitize to Olaparib*

Inhibition of PARP is an established therapeutic strategy for breast and ovarian cancer in BRCA1-deficient tumors.[29] As expected,[30] we observed an increased phosphorylation of histone H2AX, a known marker of DNA DSBs, upon treatment with olaparib in MPM spheroids (Supplementary Fig. 2), suggesting engagement of BRCA1 in DNA repair.[31,32] Because BAP1 can interact with BRCA1 and because BAP1FL reconstitution in BAP1-null cell lines leads to resistance to PARP inhibition (unlike in BAP1Δ-reconstituted cells), we analyzed the expression of BAP1Δ compared with expression of BAP1FL in MPM cell lines. Sequencing revealed the presence of WT BAP1 in all tested cell lines. We grouped them in two groups: low BAP1Δ, with a BAP1Δ/BAP1 expression ratio value of 20% or less (Mero82, ACC-Meso1, SDM103T2, H2052) and high BAP1Δ ($BAP1\Delta^{high}$), with a BAP1Δ/BAP1 expression ratio value greater than 20% (MSTO-211H, SPC111, ZL34, and Mero41) (Fig. 5*A*). The threshold value of 20% represents the median of BAP1Δ/BAP1 in the MPM cell lines. We then tested whether BRCA1 depletion mediated by phosphoinositide 3–kinase (PI3K) pathway inhibition[33] would sensitize MPM cells to PARP inhibition in MPM cell lines able to form spheroids. Spheroids were treated individually with olaparib or in combination with

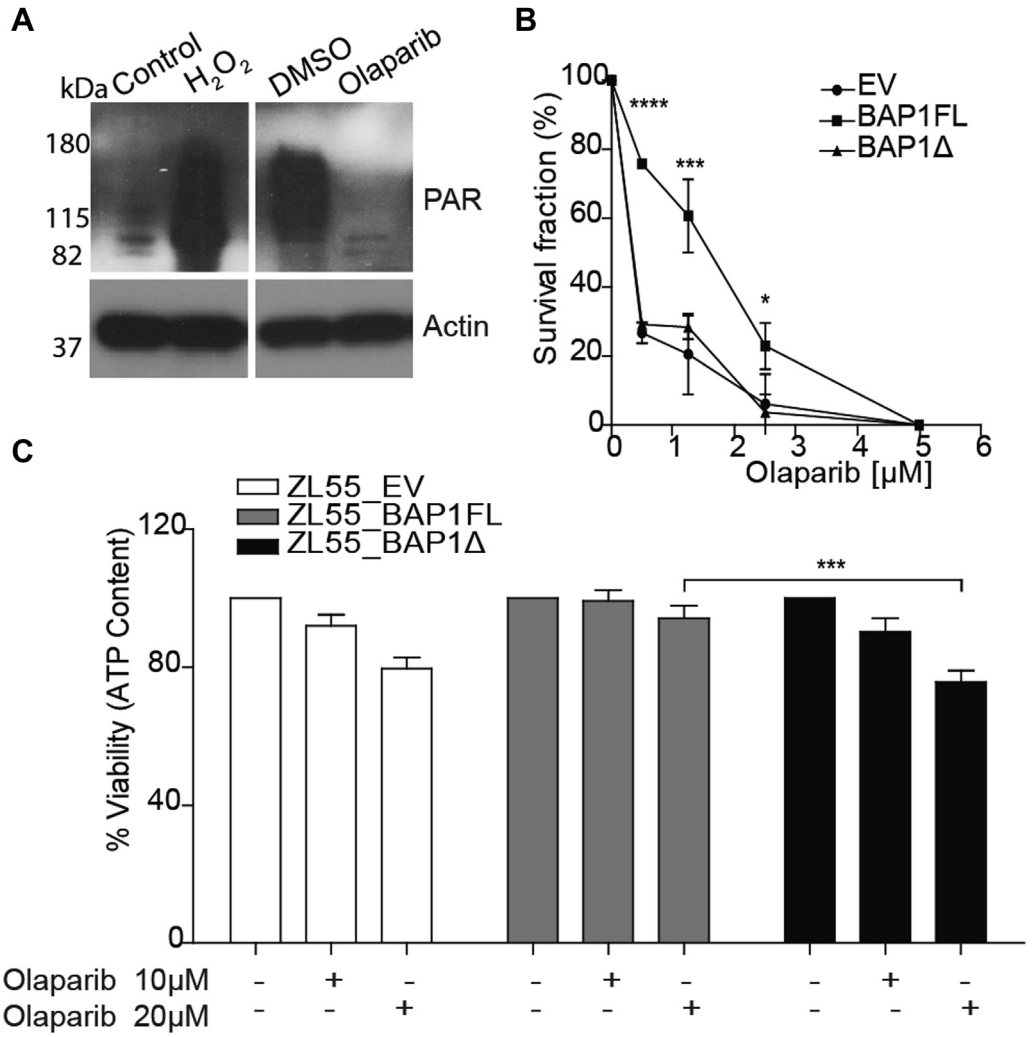


**Figure 4.** BRCA associated protein1 (BAP1) splice variant (BAP1Δ) sensitizes malignant pleural mesothelioma cells to olaparib in two- and three-dimensional in vitro cell survival assays. (*A*) Olaparib decreased poly adenosine diphosphate-ribosylation in spheroids. $H_2O_2$-induced activation of PARP1 resulting in poly adenosine diphosphate-ribosylation of proteins served as a positive control for poly(adenosine diphosphate-ribose) (PAR) immunoblot. Cells expressing BAP1Δ were as sensitive as BAP1-deficient cells to olaparib in two- (*B*) and three-dimensional (*C*) models. Results derived from three independent experiments performed in triplicate. *$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$. EV, empty vector; BAP1FL, full-length BAP1; ATP, adenosine triphosphate.

GDC0980, a dual PI3K–mammalian target of rapamycin (mTOR) inhibitor. As expected, phosphorylated protein S6 (downstream effector of PI3K-mTOR pathway) levels decreased after GDC0980 treatment in spheroids, as we had previously observed in two-dementional models[24] (Fig. 5*B*). BRCA1 expression was reduced as expected after GDC0980 treatment in the two cell lines representing the low BAP1Δ and BAP1Δ$^{high}$ groups selected on the basis of similar total BAP1 levels. Moreover, BRCA1 downregulation was intensified in the BAP1Δ$^{high}$ cell line SPC111 when treated with the combined treatment (Fig. 5*C*). BAP1Δ/BAP1 high ratio sensitized MPM cell lines to olaparib (Fig. 5*D* and *E*). As previously shown, GDC0980 decreased ATP content[24] and the BAP1Δ$^{high}$ group showed a complete ATP drop with the combined treatment (see Fig. 5*E*). Spheroids derived from the normal mesothelium cell line SDM104 were resistant to olaparib and, as previously described, were growth inhibited by GDC0980.[24] ATP content decreased further by the combined treatment, albeit to a lesser extent compared with mesothelioma cells (see Fig. 5*E*).

## Discussion

In this study we showed that expression of the BAP1Δ isoform over a threshold level, defined as 20% of total BAP1, sensitizes MPM cells expressing WT BAP1 to olaparib-induced cytotoxicity. Combining this treatment with PI3K-mTOR inhibitors, which decrease BRCA1 levels, results in stronger effects.

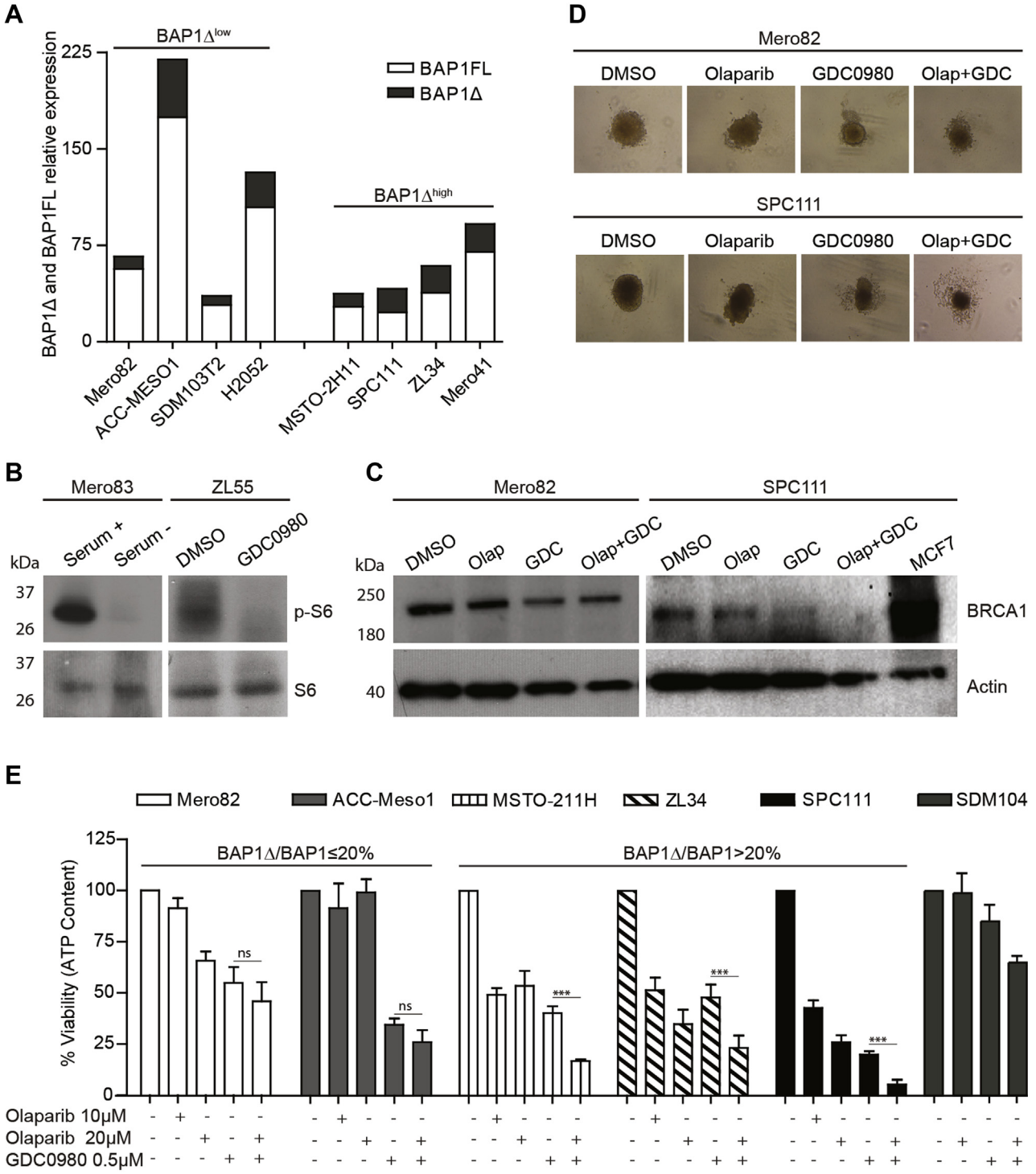


**Figure 5.** BRCA associated protein1 (BAP1) splice variant (BAP1Δ) expression level and inhibition of phosphoinositide 3-kinase–mammalian target of rapamycin sensitize malignant pleural mesothelioma (MPM) cells to olaparib. (*A*) Relative expression of BAP1Δ and full-length BAP1 (BAP1FL) in MPM. (*B*) GDC0980 decreased phosphorylation of protein S6 (p-S6) in MPM spheroids. (*C*) BRCA1 levels in three-dimensional spheroids obtained from Mero8p-s62 and SPC111 cells untreated (DMSO) and treated with 20$\mu$M olaparib or 0.5 $\mu$M GDC0980 individually or in combination. MCF7 cells lysate was included as positive control for BRCA1 expression. (*D*) Representative light micrographs of Mero-82 and SPC111 spheroids treated with 20 $\mu$M olaparib and 0.5 $\mu$M GDC0980. (*E*) Effect of combined olaparib and GDC-0980 in MPM cell lines grouped on the basis of high (BAP1$\Delta^{high}$) and low (BAP1$\Delta^{low}$) expression of BAP1Δ or in normal mesothelial cells SDM104. Results derived from three independent experiments performed in triplicate. ***$p < 0.001$. BAP1FL, full-length BAP1.

Deficiency in processes involved in DNA damage response can be exploited as a therapeutic strategy. For example, when DNA single-strand breaks occur, cells undergo PARP-mediated DNA repair. If this mechanism is inhibited (e.g., by olaparib), single-strand breaks persist and turn into DSBs. When these accumulate, they become lethal for cancer cells when specific DNA repair processes (such as homologous recombination) are impaired, as in the case of BRCA-deficient breast and ovarian cancer.[29] Interestingly, the expression of BRCA1 is essential in mediating apoptosis induced by vinorelbine (an antimitotic drug used in second-line therapy) in MPM cells,[34] and low BRCA-1 expression and vinorelbine resistance were observed in about 40% of patients with MPM. However, it remains unclear whether this is due to BAP1 loss of function (especially because recent studies have suggested a role of BAP1 in BRCA1 stabilization in mesothelioma[35]) or to some other mechanism in which BAP1Δ might be involved. Homologous recombination deficiency can also be induced by impairing BRCA1 expression through PI3K inhibition, resulting in cell sensitization to PARP inhibition.[33]

In our study we characterized a novel isoform of BAP1 that exhibits diminished deubiquitinating activity on histone H2A and is very likely unable to exert its function in DNA repair. The association between alternative splicing and cancer is rapidly emerging, because isoforms of a protein can have opposite functions.[36] The appearance of this isoform in placentals may reflect functional requirements unique to placental mammals, as is the case for other chromatin modifiers.[37] Interestingly, the alternative acceptor site for BAP1Δ scores almost as high as the one for BAP1FL, and therefore, exonic splicing enhancers[38] may possibly play a role in the differential expression. BAP1Δ was still able to enter the nucleus; therefore, a possible mechanim of action is interference with BAP1FL dimerization with ASLX1[39] and consequent inactivation. Another intriguing possibilty is inferred by the appearance of higher-molecular-weight forms of BAP1 after coexpression of BAP1FL and BAP1Δ. The higher molecular weight may represent ubiquitin conjugating enzyme E2 O-dependent ubiquitin-conjugated forms of BAP1, which accumulate especially when BAP1 cannot efficiently deubiquinate itself.[40]

Although further investigations that are beyond the scope of this study are necessary to elucidate the underlying mechanism of BAP1Δ interference with BAP1FL, we have demonstrated that expression of BAP1Δ over a certain threshold level, even in the presence of functional BAP1, could be exploited in combination with PI3K-mTOR inhibitors in a therapeutic approach using PARP inhibitors.

Potential synthetic lethality between PARP and PI3K/mTOR inhibition has been recently documented in several models,[33,41–43] and besides decreasing BRCA1 expression, other mechanisms involve nucleotide depletion.[41] Olaparib is a U.S. Food and Drug Administration–approved targeted therapy in cancer,[29] and GDC0980 has proved to have some efficacy in a clinical study, especially in patients with mesothelioma[44]; therefore, this strategy is potentially easy to implement in a clinical trial. Because BAP1 is WT in 77% of MPMs,[45] 15% of patients with MPM could potentially benefit from this therapeutic strategy.

## Acknowledgments

This work was supported by the Walter Bruckerhoff Foundation, Swiss National Science Foundation grant CRSII3 147697/1, and the Foundation for Applied Cancer Research.

## Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at http://dx.doi.org/10.1016/j.jtho.2017.03.023.

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